



Case Studies of Molecular Methods: *Method Comparisons And Source Tracking Applications*

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Comparison Study - Background

- Mathes and others (2000) found *E. coli* in 16 of 50 sampled domestic-water wells in Berkeley County, WV
- Many of these wells serve as sources of untreated drinking water
- Local authorities need help identifying the sources of fecal pollution to remedy the situation



Cooperators

VA Department of Environmental Quality
VA Dept of Conservation and Recreation
WV Department of Environmental Protection
WV Department of Agriculture
Berkeley County, WV



Problems

- In this karst groundwater system, identification or quantification of the source(s) of the fecal pollution is more difficult than performing a sanitary survey.
- Bacterial source tracking methods appear useful for identifying these sources of fecal pollution, but there remain several unresolved issues:
 - Protocols are defined in the literature but verification studies are mostly lacking
 - There is little for identifying which method is best for your specific application



BST Methods Comparison Study

- Sampling for this work is being conducted in Berkeley County, West Virginia, but the study involves researchers from across the nation and 3 different USGS district offices (Melvin Mathes of WV and Don Stoeckel of Ohio).
- Five Genotypic Methods (and investigators)
 - Ribotyping using two different enzyme sets (George Lukasik, Mansour Samadpour)
 - Pulsed-field Gel Electrophoresis (West Virginia Department of Agriculture)
 - rep-PCR using two different primer sets (Howard Kator, Don Stoeckel)
- Two Phenotypic Methods (and investigators)
 - Antibiotic Resistance Analysis (Bruce Wiggins)
 - Carbon Substrate Utilization (Chuck Hagedorn)



Methods Comparison - Study Design

- Collect fresh feces from at least 20 individuals per source across the study area.
- Isolate and confirm a library of known *E. coli* from the fresh fecal samples:
 - Total of 100 confirmed *E. coli* per source (note that three researchers received only 70 isolates per source)
 - Total known library size of 900 isolates (or 630 for three researchers)
- Prepare a blind sample set that includes:
 - A total of 200 blind isolates comprised of:
 - Replicates from the known library
 - Fresh isolates from the 9 prominent sources
 - Fresh isolates from sources that were not considered in the original known library (mice, cats, raccoons)



BST Methods Comparison Study

- Prominent sources of fecal pollution that are being considered:
 - Humans
 - Cattle (beef and dairy)
 - Chickens
 - Swine
 - Horses
 - Dogs
 - Canada Geese
 - Deer



Methods Comparison - Study Design

- Each researcher will identify the source of each isolate in the blind sample set.
- Results will be scored and the following will be considered for each method:
 - Accuracy of isolate identification
 - Precision (reproducibility of replicate isolate analyses)
 - Robustness (isolates from sources not in the library are not associated with a represented source group)
 - Practical concerns like cost and time will be considered



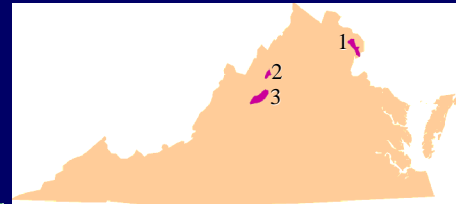
Methods Comparison - Results

- We are currently analyzing and interpreting the data from each laboratory. Results haven't been released yet or discussed with the researchers.
- In a general sense, we found that:
 - In this study, under these conditions...
 - All the methods did not produce comparable results; some methods were more successful than others.
 - We are still trying to determine why certain methods were successful or unsuccessful.
 - Results will be published this Fall.



Source Tracking Applications

1. Accotink Creek – Urban
2. Blacks Run – Mixed Urban/Agricultural
3. Christians Creek - Agricultural



Methods Comparison - Conclusions

- This is one of a very few comparisons studies being done. While it will be very informative, it cannot be considered comprehensive. We need to see what some of these other comparison studies determine.
- Without stepping through the results, we can offer these conclusions/recommendations:
 - Perform considerable QA/QC in your BST work! This may include (1) analyzing blind collections of known isolates, (2) use of multiple BST methods, and (3) the use of other tracers to support the BST work.
 - Source tracking has tremendous potential, just be cautious in your application of this new technology.



Study Objectives

Bacteria Source Tracking

- Determine the dominant sources of fecal coliform bacteria through genetic fingerprinting

Watershed Modeling

- Generate source-load reduction scenarios that meet the State's water-quality standard for fecal coliform bacteria

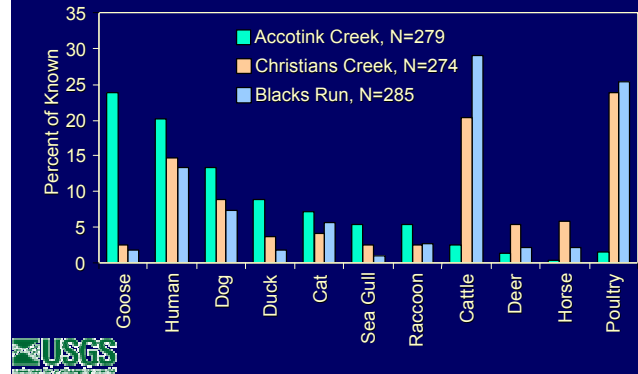


Study Design

- Important to recognize that your study design should be directly controlled by your specific study questions.
- Field Data Collection
 - Water-sample collection
 - Baseflow - 8 samples every 6 weeks (for 18 months)
 - Stormflow - 10 samples during 5 events
 - Continuum - 5 samples during 4 events
 - Source Samples
- Bacteria Source Tracking Analysis (Ribotyping)

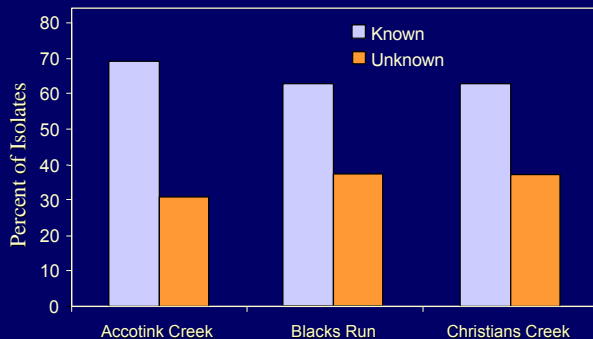
Preliminary Results of BST:

By Individual Contributor



Preliminary Results of BST:

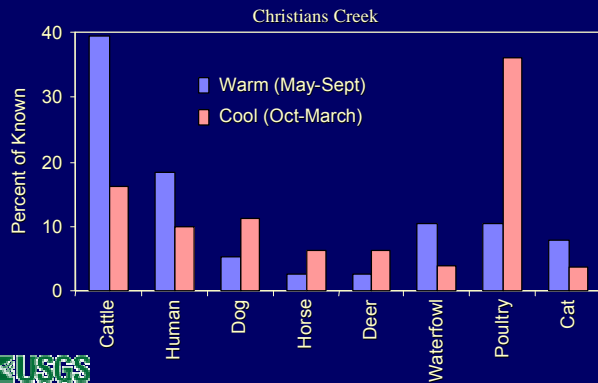
Known and Unknown Isolates



Some of our QA/QC Activities

- The results seem entirely reasonable, based on the land use practices in each watershed. We wouldn't reject the results if they weren't what we expected; rather, if the results didn't make sense, we'd reevaluate our sampling plan and our QA/QC work to establish confidence in the results.
- Performed a blind isolate matching experiment to associate single, duplicate, and triplicate copies of isolates with each other and with the known-source library.
- Performed analyses of organic compounds that are indicative of sewage (caffeine, cotinine, and such) to support the BST identification of human contributions to the streams.
- Explored the patterns of arsenic in streamwater to support the BST identification of poultry contributions to the streams.

Preliminary Results of BST: Comparison of Warm and Cool Seasons



Take Home Messages

1. In your source tracking work, be sure to perform considerable QA/QC to ensure that you have confidence in your results.
2. In designing a BST sampling program, always bear in mind that your specific question will guide your study design.



Preliminary Results of BST: Comparison of Lowflow and Stormflow Sources

